

# PATENT SPECIFICATION

DRAWINGS ATTACHED

1058,975



Date of Application and filing Complete Specification: Aug. 6, 1963.

No. 30982 63.

Application made in United States of America (No. 217013) on Aug. 15, 1962.

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Index at acceptance :—C6 EH; A5 B35; C2 C(3A12A2, 3A12B5, 3A12B6, 3A12C1, 3A12C2)

Int. Cl.:—C 12 c 3/00 // A 61 k. C 07 c

## COMPLETE SPECIFICATION

### Method of Treating Hops

5 We, S. S. STEINER, INC., a corporation  
organised and existing under the laws of the  
State of New York, United States of America,  
of 655 Madison Avenue, New York 21, New  
York, United States of America, do hereby  
declare the invention for which we pray that  
a patent may be granted to us, and the  
method by which it is to be performed, to be  
particularly described in and by the follow-  
10 ing statement:

The present invention overcomes the dis-  
advantages of the whole hop brewing pro-  
cedure with none of the concurrent prob-  
lems found in the present hop-extract pro-  
cesses. As will be readily apparent from the  
description which follows, the present inven-  
tion provides relatively pure compounds in  
a form which is readily utilizable by the  
brewer.

More particularly, the present invention is

## ERRATA

SPECIFICATION No. 1,058,975

Page 1, line 73, for "beverage" read "bever-  
age"

Page 5, line 85, after "the" insert "above"

THE PATENT OFFICE  
20th March 1967

35 produce as good a flavor as whole hops. They  
are generally in a syrupy, viscous form which  
makes it difficult to add them to the brewery  
wort and to obtain a homogeneous distribu-  
tion in the wort. In addition, these extracts  
do not remain entirely stable. Attempts have  
been made to obtain different types of extracts  
40 in order to separate components such as oils,  
tannins and acids. These extracts have not  
been wholly accepted by the brewers who  
find that the best flavor is obtained by the  
addition of whole hops rather than any of  
45 these extracts.

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separating the water soluble salts or said  
 $\alpha$ -acids from the salts of  $\beta$ -acids contained in  
hops, which comprises treating hops with a  
water-immiscible organic co-solvent for said  
 $\alpha$ -acids and  $\beta$ -acids, separating from the  
treated hops a solvent extract containing said  
 $\alpha$ -acids and  $\beta$ -acids, treating the solvent ex-  
tract with a substantially stoichiometric  
amount, based on the estimated amount of  
 $\alpha$ -acids in the extract, of an aqueous alkali  
metal hydroxide solution, and separating the  
resultant aqueous layer, which contains said  
 $\alpha$ -acids in salt form substantially free of  $\beta$ -

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## COMPLETE SPECIFICATION

### Method of Treating Hops

We, S. S. STEINER, INC., a corporation organised and existing under the laws of the State of New York, United States of America, of 655 Madison Avenue, New York 21, New York, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

This invention relates to a novel concept in the treatment of hops. The invention includes novel products obtained from hops and novel methods for producing such products.

In the present state of the art, whole or ground hops are added to the brew wort and the residues removed after the mixture is boiled for the desired length of time. This procedure is cumbersome, wasteful and time consuming. As the hops vary in their content and degree of maturity, they produce different flavors in the beer, therefore making it difficult for brewers to reproduce the desired flavor in successive batches of beer. Furthermore, it is difficult to store whole hops for any length of time without obtaining some form of deterioration.

In order to combat these disadvantages, various extracts have been made from hops. These extracts have not been satisfactory substitutes for whole hops. They do not produce as good a flavor as whole hops. They are generally in a syrupy, viscous form which makes it difficult to add them to the brewery wort and to obtain a homogeneous distribution in the wort. In addition, these extracts do not remain entirely stable. Attempts have been made to obtain different types of extracts in order to separate components such as oils, tannins and acids. These extracts have not been wholly accepted by the brewers who find that the best flavor is obtained by the addition of whole hops rather than any of these extracts.

[Price 4s. 6d.]

The present invention overcomes the disadvantages of the whole hop brewing procedure with none of the concurrent problems found in the present hop-extract processes. As will be readily apparent from the description which follows, the present invention provides relatively pure compounds in a form which is readily utilizable by the brewer.

More particularly, the present invention is concerned with providing methods for the separation of various hop components so that a separated component, particularly a product containing a high percentage of salt of alpha-acids described hereinafter, or a group of said separated components may be added to brew wort in amounts desired at a stage in the process whereby it may be utilized most efficiently and will produce the most desirable properties in the finished beer with a high level of flavor control. It is contemplated that certain separated components may be added to beer at various stage in the manufacture, in the amounts desired to produce a controlled level of aromatic and bacteriostatic properties. In short, the present invention enables one to produce a made-to-order beer, ale, or other malt beverage with respect to essentially all the properties which hops impart to malt beverages.

Broadly speaking this invention provides a method of extracting  $\alpha$ -acids from hops and separating the water soluble salts of said  $\alpha$ -acids from the salts of  $\beta$ -acids contained in hops, which comprises treating hops with a water-immiscible organic co-solvent for said  $\alpha$ -acids and  $\beta$ -acids, separating from the treated hops a solvent extract containing said  $\alpha$ -acids and  $\beta$ -acids, treating the solvent extract with a substantially stoichiometric amount, based on the estimated amount of  $\alpha$ -acids in the extract, of an aqueous alkali metal hydroxide solution, and separating the resultant aqueous layer, which contains said  $\alpha$ -acids in salt form substantially free of  $\beta$ -

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acid salts, from the solvent layer, which contains the  $\beta$ -acids. By this method a first component may be separated from hops which is stable and contains a high percentage of salts of "hop-bitter acids" which are alpha-acids.

These alpha acids are a well known constituent of hops and comprise a mixture of humulone, co-humulone and ad-humulone. Thus where used hereinafter in the specification and claims the term "alpha-acid" or " $\alpha$ -acid" is intended to refer to this mixture. These  $\alpha$ -acids can be converted by suitable techniques e.g. by application of heat into the iso-form, referred to simply hereinafter as "isohumulone."

A second component which may be extracted from hops by the method of the invention contains a high percentage of the salts of hop-bitter acids known as the beta-acids which can be recovered as a stable powder. These  $\beta$ -acids are also a well-known constituent of hops and comprise principally lupulone.

For convenience sake, in discussing hereinafter the alkali metal hydroxide used in the invention, reference will be made to sodium hydroxide. It should be understood, however, that other equivalent hydroxide materials may be employed, e.g., potassium hydroxide.

From the above remarks, it is readily apparent that by the above procedure one can preferentially isolate and obtain alpha-acids and, later, beta-acids, in the form of sodium salts. That such preferential separation may be accomplished by the mere addition of the aforesaid aqueous alkali solutions is quite surprising. The previous experience of other chemists in the treatment of a mixture of acids with a quantity of base insufficient for complete neutralization of said acids would lead one generally to anticipate the formation of the salts of both acids. This is particularly true when the two acids have similar structures. Thus, a clean separation of one of the components, as in the case at hand, is totally unexpected. On the contrary, however, it has been found that if the sodium hydroxide is in a stoichiometric amount based on the estimated alpha-acids, there results formation of alpha-acid salts substantially free of beta-acid salts. When the resulting hexane solution is separated from the alpha-acid salts and treated with an aqueous alkali solution containing sodium hydroxide, preferably in a stoichiometric amount based on the estimated beta-acids, there results the formation of beta-acid salts substantially free of alpha-acid salts.

The general method referred to hereinabove will now be described in greater detail, reference being made to the flow sheet illustrated in the accompanying drawing.

### I Solvent Extraction

Hops are treated with a solvent capable of extracting alpha- and beta-acids. Preferably, the hops are in ground form to effect more efficient extraction of the desired components. The preferred solvent for this purpose is hexane. Other solvents may be used such as toluene and chloroform. For convenience sake, the method will be described employing hexane as the solvent. Accordingly, the first stage is a hexane extraction stage 1, as shown in the accompanying flow sheet. By this hexane extraction step there are produced two fractions, a hexane extract and a residue. The residue 2 and hexane extract 3 are then separated in any suitable manner, such as by filtration.

### II Residue Treatment

The residue 2 containing water-soluble components is treated with hot water in a water extraction stage 4, whereby the water-soluble tannins 5 are extracted therefrom. The tannins are then recovered by freeze drying in a drying stage 6, or an equivalent technique whereby possible heat labile components of the tannings are not affected. The dried tannins 7 are in stable form and are available for inclusion in appropriate amounts in tailor-made mixtures for use by the brewer in the manner described hereinbefore.

### III Hexane Extract Treatment

The amount of alpha-acids and beta-acids in the hexane extract 3 is then estimated employing any standard test method for this purpose. The test method actually employed is the spectrophotometric method described in "METHODS OF ANALYSIS OF THE AMERICAN SOCIETY OF BREWING CHEMISTS," 6th Revised Edition, 1958, pages 138-A, 138-B, 138-C.

The hexane extract is then treated with successive alkali treatments as follows:

#### (a) First Alkali Treatment

The hexane extract is treated in an alkali treatment stage 8 with an aqueous alkali solution containing sodium hydroxide in a stoichiometric amount based on the estimated amount of alpha-acids. This results in a mixture of (a) an aqueous alkali extract 9 containing the alpha-acid sodium salts, and (b) a hexane solution 10 substantially free of alpha-acids. The alkali extract 9 is then separated from the hexane solution by any suitable technique, e.g., use of a separatory funnel, and the extract 9 is dried in a drying stage 11 in order to obtain a powder 12 containing a high percentage of alpha-acid sodium salts by freeze drying. A freeze drying technique is employed since the salts are unstable to heat. Another suitable drying technique that may be employed is evaporation under vacuum.

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The dried alpha-acid sodium salts 12 are in stable form and are available for addition in appropriate amounts to the wort at an appropriate stage of the brewing process.

#### (b) Second Alkali Treatment

The hexane solution 10 (with alpha-acids removed) is then treated in a second alkali treatment stage 13 with an aqueous alkali solution containing sodium hydroxide, preferably in a stoichiometric amount based on the estimated amount of beta-acids present in the hexane extract, in such concentration as to remove from the hexane solution beta-acids in the form of sodium salts. This results in a mixture of (a) an aqueous alkali extract 14 containing the beta-acid sodium salts, and (b) a hexane solution 15 substantially free of alpha-acids and beta-acids. The alkali extract 14 is then separated from the hexane solution by any suitable technique, e.g., use of a separatory funnel, and the alkali extract is dried in a drying stage 16 in order to obtain a powder 17 containing a high percentage of beta-acid sodium salts. The same drying technique may be used as employed in obtaining the alpha-acid powder described hereinbefore.

The dried beta-acid sodium salts are in stable form and are available for addition, in appropriate amounts, to the wort or beer at an appropriate stage of the brewing process.

#### IV Hexane Solution Treatment

The hexane solution is then fractionally distilled in a distillation stage 18 whereby hop oils 19 and hexane 20 are recovered. The hop oils are available for inclusion in appropriate amounts in tailor-made mixtures for use by the brewer in the manner described hereinbefore.

If so desired, the free acids of the alpha-acid salts and beta-acid salts produced in accordance with this invention may be obtained by neutralizing the salts. More particularly, as shown in the accompanying flow sheet, the alpha-acid salts 12 and beta-acid salts 17 may be neutralized by acidification to give the free alpha-acids 12' and free beta-acids 17' which may themselves be used after having been brought to a stable form.

In determining stoichiometric amount in the examples which follow, we have taken into account the complexity of the mixture known as alpha-acids and the presence of small quantities of extraneous acidic materials. Accordingly, we have based the stoichiometric amount of sodium hydroxide on the molecular weight of humulone and have added up to 10% in excess of sodium hydroxide.

Examples V, VI, X and XI are presented by way of comparison.

#### EXAMPLE I

1,000 grams of dried hops (7.41% alpha-acids, 5.53% beta-acids by A.S.B.C. assay) were ground to a powder and extracted with hexane by stirring several hours and filtering. This process was twice repeated and the combined hexane extracts were distilled in vacuum to a volume of 3,000 ml. The solution contained 5.5% solids (a total of 166.5 grams). The solids assayed 45.60% alpha-acids and 29.17% beta-acids by A.S.B.C. assay.

180 ml. of the above hexane solution contain 10 grams of total solids, 4.56 grams of alpha-acids and 2.91 grams of beta-acids. The amounts of normal sodium hydroxide equivalent to these acids are:

Alpha-acids	12.6 ml.
Beta-acids	7.0 ml.

A) to the 180 ml. of hexane solution there were added 13 ml. of normal sodium hydroxide and 25 ml. of water, the mixture stirred at 25°C. for 10 minutes, separated, and the hexane layer was washed with 10 ml. of water. The combined water extracts were freeze dried by freezing in a dry ice-acetone bath and drying under a high vacuum.

E) To the hexane solution remaining from extraction A were added 7.0 ml. of normal sodium hydroxide and 15 ml. of water and the extraction, washing, separation and freeze drying conducted as described under A.

C) to the hexane solution remaining from extraction B there were added 7.0 ml. of normal sodium hydroxide and 15 ml. of water and the process described under A repeated.

The following table summarizes the results:

	WEIGHT DRY	ALPHA-ACIDS	BETA-ACIDS	% RECOVERY ALPHA-ACIDS	% RECOVERY BETA-ACIDS
A	5.0 Grams	83.4	0	91.4	
B	2.6 "	0	80.0		71.5
C	1.2 "	0	42.6		17.5
TOTAL	8.8 "			91.4	89.0

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This example demonstrates the sharpness of the separation obtainable by the newly conceived procedure, also the fact that the yields are excellent, particularly in the case of the alpha-acids.

## EXAMPLE II

180 ml. of the hexane solution as prepared for Example I were extracted with portions of dilute sodium hydroxide as in Example I, but the extractions were conducted at a temperature of 45°C. Results are summarized in the following table :

	WEIGHT DRY	ALPHA-ACIDS	BETA-ACIDS	% RECOVERY ALPHA-ACIDS	% RECOVERY BETA-ACIDS
A	5.0 Grams	87.07	0	95.2	
B	2.85 „	0	71.66		70.0
C	1.3 „	0	16.24		7.2
TOTAL	9.15 „			95.3	77.2

At 45°C. the yield and purity of the alpha-acids were superior to those at 25°C. This is partly due to the greater ease of separation of the two layers at the higher temperature. The total recovery of beta-acids is smaller at the higher temperature. There is little or no isomerization of alpha-acids to isohumulones under the conditions of this experiment.

for the previous examples was evaporated to dryness under high vacuum. The 10 grams of material thus obtained were dissolved in 180 ml. of naphtha. On this solution the extractions with aliquots of dilute alkali were conducted as in Example I, but the entire extraction operations were carried out at a temperature of 90°C. The resultant solutions were freeze dried as before. The following table summarizes the results :

## EXAMPLE III

180 ml. of the hexane solution as prepared

	WEIGHT DRY	ALPHA-ACIDS	BETA-ACIDS	% RECOVERY ALPHA-ACIDS	% RECOVERY BETA-ACIDS
A	4.8 Grams	45.6	0	48	
B	1.7 „	0	23.6		13.7
C	2.2 „	0	33.2		25.1
TOTAL	8.7 „			48	38.8

As the above figures show, there has been no loss of weight of material but there has been considerable loss of alpha- and beta-acids. The determinations of alpha- and beta-acids in the above, as in all assays in the Examples, has been conducted by the official A.S.B.C. method. The spectrophotometric curves as obtained by the A.S.B.C. technique indicated that isohumulones were present. Rigby and Bars (A.S.B.C. proceedings, 1961, 46-50) described a method of determining alpha-acids and isohumulones. Their method applied to fraction A yielded the following results :

metric curves as obtained by the A.S.B.C. technique indicated that isohumulones were present. Rigby and Bars (A.S.B.C. proceedings, 1961, 46-50) described a method of determining alpha-acids and isohumulones. Their method applied to fraction A yielded the following results :

	WEIGHT GRAMS	ALPHA-ACIDS	% ISO-HUMULONES	% RECOVERY ALPHA-ACIDS AND ISOHUMULONES
A	4.8	52.1	22.7	84.2

What the above figures indicate is that much isomerization has taken place during the comparatively short period of extraction at 90°C. This experiment is, in part, an isomerization of alpha-acids under buffered conditions in which the beta- and other hop-acids act as buffers to prevent over-alkalinization. This experiment also shows that alpha-acids con-

taining isohumulones may be freeze dried as their sodium salts without decomposition.

## EXAMPLE IV

300 grams of ground hops were extracted three times with hexane by stirring at room temperature and filtering each time. The extracts were combined and distilled in a

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vacuum to a volume of 1,000 ml.

Estimated alpha-acids according to A.S.B.C. assay of the hops: 22.23 grams which is equivalent to 60 ml. of normal sodium hydroxide.

The hexane solution was stirred ten minutes with 50 ml. of normal sodium hydroxide plus 50 ml. of water. The aqueous portion was separated and the extraction of the hexane repeated with 16 ml. of normal sodium hydroxide plus 20 ml. of water. The aqueous portion was separated and the hexane washed with 40 ml. of water. The aqueous portions were combined, filtered and freeze dried.

The weight of dry material was 26.0 grams A.S.B.C. assay:

Alpha-acids	79.5 %
Beta-acids	0.4 %

This represents a 93 % recovery of the alpha-acids present in the hops.

The sodium salt as obtained above was dissolved in water, the solution acidified and then extracted with ether and the ether extract dried. A yield of 93.7 % of free acid was obtained as a thick oil. This material had a specific rotation of  $-180^\circ$ . Humulone displays a specific rotation of  $-225^\circ$ . Thus, the optical rotation and the spectrophotometric assay are substantially in agreement.

The amount of sodium present in the sodium salt as determined by assay was 6.04 %, which agrees well with the 93.2 % of free acid obtained.

The hexane solution which has been extracted with alkali was further extracted with dilute sodium hydroxide until no extractable material remained in the hexane. The hexane solution was washed free of alkali with water and then distilled through a fractionating column to a volume of 60 ml. The 60 ml. was then concentrated in a vacuum until of constant weight. The material thus obtained was extracted with 90 % methanol and the methanol extract was concentrated in a vacuum of 20 ml. or less to yield 2 grams of a thick oil suitable for imparting a hop aroma to beer, either as such or after further treatment.

The original hops which had been extracted with hexane were dried in air to remove hexane and were then extracted by treating with 600 ml. of hot water, holding at  $65^\circ\text{C}$ . for 16 hours, filtering and washing with hot

water. (The temperature was held at  $65^\circ\text{C}$ . to prevent microbiological growth). The total water extract thus obtained was freeze dried to yield 52.8 grams. This material contains all of the water-soluble hop tannins and represents a useful additive to wort to produce the so-called "hot break."

#### EXAMPLE V

180 ml. of the hexane solution as prepared for Example I and containing 4.56 grams of alpha-acids and 2.91 grams of beta-acids (A.S.B.C. assay) were treated with an amount of dilute sodium hydroxide equivalent to all of the hop acids present. Thus the hexane solution was stirred for 10 minutes with 27 ml. of normal sodium hydroxide and 55 ml. of water at  $25^\circ\text{C}$ . The aqueous portion was separated, filtered and freeze dried.

Weight of dry material — 8.2 grams (A.S.B.C. assay)

Alpha-acids	34 %
Beta-acids	31 %

This represents a recovery of only 61 % of the original alpha-acids and a recovery of 89 % of the beta-acids. In Example I, wherein the same total quantity of sodium hydroxide was used, the recovery of the alpha-acids was 91.4 %. This demonstrates that considerable decomposition of alpha-acids takes place in the presence of alkali. The assay does not show any significant quantity of isohumulones to be produced under the experimental conditions. Obviously, no separation of alpha-acids from beta-acids has taken place.

#### EXAMPLE VI

360 ml. of a hexane extract of hops containing (according to the A.S.B.C. assay) 8.88 grams of alpha-acids and 6.63 grams of beta-acids were treated by stirring with a portion of dilute sodium hydroxide equivalent to 2.7 grams of alpha-acid, separating and repeating with additional similar portions of dilute sodium hydroxide to a total of four extractions. The aqueous extracts were separately freeze dried.

For each extraction 8 ml. of normal sodium hydroxide and 20 ml. of water were used. The dried samples were assayed by the A.S.B.C. method. The following table summarizes the results :

	pH OF SOLUTION	WEIGHT DRY	% ALPHA-ACIDS	% BETA-ACIDS	% RECOVERY OF ALPHA-ACIDS
A	8.15	2.0	89.1	0	20.0
B	8.5	3.3	88.2	0	32.7
C	9.25	3.4	72.7	0	27.9
D	10.5	3.2	30.0	53.5	10.8
TOTAL		11.9			91.4

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This experiment demonstrates that alpha-acids are selectively extracted when small increments of alkali are used. It will be noticed that the pH of the extracts varies over a fairly wide range without yielding any indication of the relative percent of alpha-acid being extracted. It has been demonstrated in Examples 1, 2 and 4 that over 90% of the alpha-acids and none of the beta-acids (as shown by the A.S.B.C. assay) can be extracted

by use of the proper quantity of sodium hydroxide.

#### EXAMPLE VII

180 ml. of the hexane extract of hops as prepared for Example 4 were extracted with portions of dilute alkali as in Example 1, except that normal potassium hydroxide was used in place of sodium hydroxide.

The results were as follows :

	WEIGHT GRAMS	% ALPHA-ACIDS	% BETA-ACIDS	% RECOVERY ALPHA	% RECOVERY BETA
A	5.3	76.4	0	80.0	
B	2.8	8.8	59.3	5.4	57.0
C	1.5	0.0	40.8		20.7
TOTAL	9.6			85.4	77.7

This experiment demonstrates that potassium hydroxide is slightly less efficient than sodium hydroxide in separating alpha- and beta-acids under equivalent conditions.

180 ml. of a hexane extract of hops as prepared for Example 1 were extracted with portions of dilute sodium hydroxide in the same manner as in Example 1, but the extractions were conducted at 65°C. The results were as follows :

#### EXAMPLE VIII

	WEIGHT GRAMS	% ALPHA-ACIDS	% BETA-ACIDS	% RECOVERY ALPHA	% RECOVERY BETA
A	4.9	75.5	0	79.0	
B	2.2	3.27	44.18	1.5	54.8
C	1.2	0	56.17		38.0
TOTAL	8.3			80.5	92.8

As the above experiment shows, the recovery of alpha-acids is not as good at 65°C. as at 45°C. The spectrophotometric curves indicate that about 10% of the alpha-acids originally present have been converted to isohumulones.

The laboratory treated wort showed :  
45.6 ppm. isohumulones  
16.0 ppm. alpha-acids  
The brewery treated wort showed :  
28.3 ppm. isohumulones  
10.4 ppm. alpha-acids

#### EXAMPLE IX

To 1,000 ml. of a routine unhepped brewers wort, there were added 100 milligrams of the material obtained as described in Example 4. According to the A.S.B.C. assay, this contained 79.5 milligrams of alpha-acids and 0.4 milligrams of beta-acids, and is the equivalent of 1,160 milligrams of hops per liter of wort (or of 0.3 pounds of hops per barrel of wort). The treated wort was boiled under reflux for 5 hours, cooled, and a filtered sample assayed according to the Rigby and Bars procedure (A.S.B.C. proceedings 1961, 46-50).

A sample of similar wort treated with 0.3 pounds of hops per barrel of wort and boiled, at the brewery was analyzed in the same manner.

The aforedescribed example demonstrates that the loss of bittering agents in the transition from hops to wort is considerably smaller when the alpha-acids have been extracted by the procedure outlined in accordance with this invention.

#### EXAMPLE X

In Example 3 it was noted that isomerization had taken place and fraction A contained 22.7% isohumulones according to the Rigby assay, as well as 52.1% alpha-acids by the A.S.B.C. assay.

3.0 grams of fraction A obtained in the experiment described in Example 3 (containing 1.563 grams of alpha-acids and 0.681 grams of isohumulones) were dissolved in water, acidified with hydrochloric acid and extracted with three portions, each 50 ml.,

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of hexane. The hexane solution thus obtained was freed of hydrochloric acid by shaking with water and separating. The hexane extract was stirred 10 minutes with 3.3 ml. of normal sodium hydroxide plus 10 ml. of water. After separation, the hexane was washed with 5 ml. of water. The combined aqueous portions were filtered and freeze dried.

with another portion of 3.3 ml. of normal sodium hydroxide and 10 ml. of water plus the use of 5 ml. of water for washing. The combined extracts were also filtered and freeze dried.

The quantity of sodium hydroxide used in each extraction was equivalent to 1.2 grams of alpha-acids (or isohumulones).

Following is the analysis of the dried sample.

WEIGHT GRAMS	% ALPHA-ACIDS A.S.B.C. ASSAY	% ISOHUMULONES RIGBY ASSAY
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1	1.2	26.83	37.70
2	1.2	54.50	11.46

This experiment indicates that the isohumulones are preferentially extracted by dilute sodium hydroxide but the separation is not sharp, as shown by the fact that some isohumulones are present in fraction 2.

1.46% isohumulones by Rigby assay  
A portion of 130 ml. of the above solution (containing 2.66 grams of alpha-acids according to the A.S.B.C. assay and 2.63 grams of isohumulones according to the Rigby assay) was stirred 10 minutes with 8 ml. of normal sodium hydroxide and 15 ml. of water, separated, and the hexane solution washed with 5 ml. of water. The aqueous portions were combined, filtered and freeze dried. The hexane solution was then treated in a similar fashion with another 8 ml. portion of normal sodium hydroxide.

The analytical results were as follows :

WEIGHT GRAMS	% ALPHA-ACIDS A.S.B.C. ASSAY	% ISOHUMULONES RIGBY ASSAY
--------------	------------------------------	----------------------------

A	2.1	9.0	55.1
B	3.8	40.9	19.2

This experiment demonstrates that mixtures of alpha- and isohumulones can be separated but not in the precise manner with which mixtures of alpha- and beta-acids can be separated.

It is seen, therefore, that this invention relates to isolation of alpha-acids from beta-acids from a solvent extract containing both acids, based on combining with said extract, an aqueous alkali solution containing an alkali metal hydroxide, preferably sodium hydroxide, in a substantially stoichiometric amount based on an estimated amount of alpha-acids present in the solvent extract. In general, a single alkali extract treatment is employed for isolating the alpha-acids, i.e., the alkali metal hydroxide is in an amount approximating the aforementioned stoichiometric amount. This does not exclude, however, incremental isolation of the alpha-acids by employing a number of alkali extract treatments wherein the alkali metal hydroxide present for each treatment is less than the stoichiometric amount. When such technique

is used, the total of alkali metal hydroxide used in the plurality of alkali extraction steps should not exceed the stoichiometric amount if one wishes to isolate only alpha-acids.

It should be understood that this invention includes techniques for isolation of alpha-acids, isohumulones, or mixtures of alpha-acids and isohumulones. As described hereinbefore in detail, whether or not alpha-acids alone are isolated, or some isohumulones are also present, depends upon the temperature of the alkali extract treatment. In general, temperatures up to about 50°C. give only the alpha-acids, while at higher temperatures, e.g., 60°C. or above, mixtures of alpha-acids and isohumulones are obtained.

As indicated hereinbefore, the hop components obtained in accordance with the invention are used in the brewing of malt beverages. While the present invention is primarily concerned with the brewing of alcoholic malt beverages, the components have other uses, such, for example, as for brewing of non-alcoholic malt beverages.

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When used in the brewing of malt beverages, alcoholic or non-alcoholic, the alpha-acids and beta-acids are added to the brewing either in the powder form, i.e., the alkaline salt form, or in the oil form, i.e., the free acid form obtained by acidification of the salt. The free acids may be brought to powder form by absorption on inert materials. The free acids may be encapsulated with suitable materials, such as maltodextrin. If so desired, the hop oils and tannins obtained in accordance with this invention may also be added to the brewing wort.

The alpha-acids, in the form of salts or free acids, may also be added to food compositions as flavouring additives. Then, too, the beta-acids, which possess bacteriostatic properties, in the form of salts or free acids, may be added to compositions, e.g., beer, wherein the inhibition of bacteria is desired. The hop oils may be added to compositions, e.g., perfumes, for the purpose of imparting thereto an aromatic aroma. In connection with the salt mixtures, it should here be mentioned that the mixture of alpha-acid salts and salts of isohumulones *per se* forms the subject matter of our copending application No. 35222/66 (Serial No. 1058975) which is divided herefrom.

The principles of this invention are applicable to any form of hops normally used in the brewing art. Examples of such forms are dried hops (ground or whole), fresh hops (green Hops), frozen hops and lupulin (dried or fresh).

#### WHAT WE CLAIM IS :—

1. A method of extracting  $\alpha$ -acids (as hereinbefore defined) from hops and separating the water soluble salts of said  $\alpha$ -acids from the salts of  $\beta$ -acids (as hereinbefore defined) contained in hops, which comprises treating hops with a water immiscible organic co-solvent for said  $\alpha$ -acids and  $\beta$ -acids, separating from the treated hops a solvent extract containing said  $\alpha$ -acids and  $\beta$ -acids, treating the solvent extract with a substantially stoichiometric amount, based on the estimated amount of  $\alpha$ -acids in the extract, of an aqueous alkali metal hydroxide solution, and separating the resultant aqueous layer, which contains said  $\alpha$ -acids in salt form substantially free of  $\beta$ -acid salts, from the solvent layer, which contains the  $\beta$ -acids.

2. A method according to Claim 1, in which the aqueous alkali metal hydroxide solution used is an aqueous sodium hydroxide solution.

3. A method according to Claim 1 or 2, in which the co-solvent for the  $\alpha$ -acids and  $\beta$ -acids is hexane.

4. A method according to Claim 1, 2 or 3 in which the treatment of the solvent extract with the aqueous alkali metal hydroxide solution is conducted at a temperature which is

low enough to prevent isomeric conversion of the  $\alpha$ -acid salts into the iso form.

5. A method according to Claim 4, including the steps of removing the water from the aqueous solution of  $\alpha$ -acid salts and recovering the  $\alpha$ -acid salts in dry powder form.

6. A method according to Claim 5, in which the water is removed by freeze-drying.

7.  $\alpha$ -Acid salts in dry powder form, when prepared by a method claimed in Claim 5 or 6.

8. A method according to Claim 4, including the steps of neutralizing the aqueous solution of  $\alpha$ -acid salts and recovering the  $\alpha$ -acids in free form.

9.  $\alpha$ -Acids in free form, when extracted by a method according to Claim 8.

10. A method according to Claim 1, 2 or 3, modified in that the treatment of the solvent extract with the aqueous alkali metal hydroxide solution is conducted at an elevated temperature sufficient to cause at least partial isomeric conversion of the  $\alpha$ -acid salts to salts of isohumulone.

11. A method according to Claim 10, including the steps of removing the water from the aqueous solution of  $\alpha$ -acid salts and recovering, in dry powder form, a mixture of  $\alpha$ -acid salts and salts of isohumulone.

12. A method according to Claim 11, in which the water is removed by freeze-drying.

13. A mixture of  $\alpha$ -acid salts and salts of isohumulone in dry powder form, when prepared by a method according to Claim 11 or 12.

14. A method according to Claim 10, including the steps of neutralizing the aqueous solution of  $\alpha$ -acid salts and iso salts and recovering a mixture of free  $\alpha$ -acids and isohumulone.

15. A mixture of  $\alpha$ -acids and isohumulone in free acid form, when prepared by a method according to Claim 14.

16. A method according to any one of Claims 1-6, 8, 10-12 and 14, including the further steps of treating the solvent layer containing  $\beta$ -acids with a further quantity of an aqueous alkali metal hydroxide solution thereby to form a second aqueous layer which contains said  $\beta$ -acids in salt form and a solvent layer, and separating said second aqueous layer from the solvent layer.

17. A method according to Claim 16, in which the further alkali metal hydroxide solution is a solution of sodium hydroxide.

18. A method according to Claim 16 or 17, in which the further alkali metal hydroxide solution is used in a stoichiometric amount based on the estimated amount of  $\beta$ -acids present.

19. A method according to any one of Claims 16-18, including the further steps of removing the water from the aqueous solution containing the  $\beta$ -acid salts and recovering the  $\beta$ -acid salts in dry powder form.

20. A method according to Claim 19, in

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which the water is removed by freeze drying.

21. Alkali metal salts of  $\beta$ -acids in dry powder form, when prepared by a method according to claim 19 or 20.

5 22. A method according to any one of Claims 16-18, including the further steps of neutralizing the aqueous solution of  $\beta$ -acid salts and recovering the  $\beta$ -acids in free form.

10 23.  $\beta$ -Acids in free form, when prepared by a method claimed in Claim 22.

15 24. A method according to any one of Claims 16-20 and 22, including the further step of distilling the remaining solvent layer to remove the solvent and to recover therefrom a hop oil extract.

25. A hop oil extract when prepared by a method claimed in Claim 24.

20 26. A method according to any one of Claims 1-6, 8, 10-12, 14, 16-20 and 22, including the further step of treating the hop residue from the solvent extraction step with water to extract water soluble tannins therefrom and recovering the water soluble tannins.

25 27. Water soluble tannins when prepared by a method according to Claim 26.

28. A method of brewing a malt beverage which comprises adding to the brewing wort, as a flavouring agent, one or more of the following, viz, an alkali metal salt as claimed in Claim 7, an  $\alpha$ -acid as claimed in Claim 9, a mixture as claimed in Claim 13, a mixture as claimed in Claim 15, an alkali metal salt as claimed in Claim 21, a  $\beta$ -acid as claimed in Claim 23, a hop oil extract as claimed in Claim 25, or a water-soluble tannin as claimed in Claim 27.

29. A method of inhibiting bacterial degradation of beer which comprises adding thereto a  $\beta$ -acid according to Claim 23 or a water soluble salt thereof.

30. A method of improving the aroma of beer which comprises adding thereto a hop oil extract according to Claim 25.

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## COMPLETE SPECIFICATION

1 SHEET

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